Gábor Harmati[1], Ferenc Papp[2], Norbert Szentandrássy[1], László Bárándi[1], Ferenc Ruzsnavszky[1], Balázs Horváth[1], Tamás Bányász[1], János Magyar[1], György Panyi[2], Zoltán Krasznai[2], Péter P. Nánási[1]

Effects of the PKC inhibitor chelerythrine and bisindolylmaleimide I (GF-109203X) on delayed rectifier K^+ currents

[1]Department of Physiology, University of Debrecen, H-4012 Debrecen, P.O.Box 22, Hungary

[2]Department of Biophysics and Cell Biology, University of Debrecen, H-4012 Debrecen, P.O.Box 39, Hungary

Correspondence:

Péter P. Nánási at the above address

Phone/FAX: +36-52-255-575/+36-52-255-116

E-mail: nanasi@phys.DOTE.hu

Running title: PKC inhibitors and cardiac K^+ currents

Abstract

PKC inhibitors are useful tools for studying PKC-dependent regulation of ion channels. For this purpose high PKC specificity is a basic requirement excluding any direct interaction between the PKC inhibitor and the ion channel. In the present study the effects of two frequently applied PKC inhibitors, chelerythine and bisindolylmaleimide I, were studied on the rapid and slow components of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}) in canine ventricular cardiomyocytes and on hERG channels expressed in HEK cells. Whole cell version of the patch clamp technique was used in all experiments. Chelerythrine and bisindolylmaleimide I (both 1 μM) suppressed I_{Kr} in canine ventricular cells. This inhibition developed rapidly suggesting a direct drug-channel interaction. In HEK cells heterologously expressing hERG channels, chelerythrine and bisindolylmaleimide I blocked hERG current in a concentration-dependent manner, having EC_{50} values of 0.11±0.01 and 0.76±0.04 μM, respectively. Both chelerythrine and bisindolylmaleimide I strongly modified gating kinetics of hERG: voltage-dependence of activation was shifted towards more negative voltages and activation was accelerated. Deactivation was slowed by bisindolylmaleimide I but not by chelerythrine. I_{Ks} was not significantly altered by bisindolylmaleimide I and chelerythrine. No significant effect of 0.1 μM bisindolylmaleimide I or 0.1 μM PMA (PKC activator) was observed on I_{Kr} arguing against significant contribution of PKC to regulation of I_{Kr}. It is concluded that neither chelerythrine, nor bisindolylmaleimide I is suitable for selective PKC blockade due to their direct blocking actions on the hERG channel.

Keywords: Chelerythrine - bisindolylmaleimide I - Protein kinase C - Delayed rectifier K^+ current - hERG channel - Dog myocytes

Introduction

Delayed rectifier K^+ currents play pivotal role in repolarization of the cardiac action potential. In the ventricular myocardium of most mammalian species, including dog and human, delayed rectifier K^+ current is composed of two independent components, called I_{Kr} and I_{Ks} (Gintant 1996, Li et al. 1996). Both components are known to be under the control of the cAMP/PKA pathway, while the role of PKC in regulation of I_{Kr} and I_{Ks} is controversial, and therefore, poorly understood in spite of the extensive investigations of the field (Tohse 1990, Kathöfer et al. 2003, Xiao et al. 2003, Thomas et al. 2003, Thomas et al. 2004a, Toda et al. 2007, Matavel and Lopes 2009). Appropriate approach of this problem might be the application of selective PKC inhibitors, provided that these agents fail to interact directly with the ion channels mediating delayed rectifier K^+ currents. These conditions, however, have never been controlled, except for an early study demonstrating that I_{Kr} was directly blocked by bisindolylmaleimide I in guinea pig ventricular cells (Thomas et al. 2004b). The aim of the present work was to study the effects of two frequently used PKC inhibitors, chelerythrine and bisindolylmaleimide I, on the rapid and slow components of the delayed rectifier K^+ current (I_{Kr} and I_{Ks}) in ventricular cardiomyocytes of the dog, a species having action potential characteristics and properties of the underlying transmembrane ion currents most resembling the human (Szentandrássy et al. 2005, Szabó et al. 2005). Direct effects of the compounds were tested in an expression system containing hERG channels not coupled to signal

transduction pathways. It was found that both chelerythrine and bisindolylmaleimide I caused direct blockade of I_{Kr} – but not I_{Ks} – indicating that these drugs are not suitable to study the contribution of the PKC system to physiological regulation of I_{Kr}.

Materials and methods

Experiments with isolated canine cardiomyocytes

Single canine ventricular myocytes were obtained from hearts of adult beagle dogs using the segment perfusion technique (Magyar et al. 2000). The animals (10-14 kg) were anesthetized with i.v. injection of 10 mg/kg ketamine hydrochloride (Calypsol, Richter Gedeon, Hungary) + 1 mg/kg xylazine hydrochloride (Sedaxylan, Eurovet Animal Health BV, The Netherlands). After opening the chest the heart was rapidly removed and the left anterior descending coronary artery was perfused using a Langendorff apparatus. Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle, Joklik modification, Sigma), supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaH_2PO_4 (200 mg/l), was used during the initial 5 min of perfusion to remove Ca^{2+} and blood from the tissue. After addition of $NaHCO_3$ (1.3 g/l) the pH of this perfusate was adjusted to 6.9 by equilibrating the solution with a mixture of 95% O_2 and 5% CO_2. Cell dispersion was performed for 30 min in the same solution containing also collagenase (660 mg/l, Worthington CLS-II), bovine albumin (2 g/l) and $CaCl_2$ (50 μM). During the isolation procedure the solutions were gassed with carbogen and the temperature was maintained at 37 ^{o}C. The cells were rod shaped and showed clear striation when the external calcium was restored.

I_{Kr} and I_{Ks} were recorded at 37 ^{o}C from Ca^{2+}-tolerant canine ventricular cells superfused with Tyrode solution containing (in mM) NaCl 140, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES 5, glucose 10, at pH 7.4. This superfusate was supplemented with 5 μM nifedipine plus 1 μM E-4031 when measuring I_{Ks}, or 5 μM nifedipine plus 1 μM HMR-1556 in case of recording I_{Kr}, in order to eliminate L-type Ca^{2+} current, I_{Kr}, or I_{Ks}, respectively. Suction pipettes, fabricated from borosilicate glass, had tip resistances of 1.5-2 MΩ after filling with pipette solution composed of (in mM) K-aspartate 100, KCl 45, $MgCl_2$ 1, HEPES 5, EGTA 10, K-ATP 3. The pH of this solution was adjusted to 7.2 with KOH. Membrane currents were recorded with an Axopatch-200B amplifier (Axon Instruments) using the whole cell configuration of the patch clamp technique. After establishing high (1-10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1 ms. Ion currents were normalized to cell capacitance, determined in each cell using short hyperpolarizing pulses from -10 mV to -20 mV. The series resistance was typically 4-8 MΩ before compensation (usually 50-80%) prior to the measurement. Experiments were discarded when the amplitude of I_{Kr} or I_{Ks} was unstable within the initial 5 min of the experiment, or the series resistance was high or increased during the measurement. Outputs from the clamp amplifier were digitized at 20 kHz using an A/D converter (Digidata-1200, Axon Instruments) under software control (pClamp 6.0, Axon Instruments).

hERG current measurements in transfected human embryonic kidney cells

hERG channels were expressed in a stable manner in a HEK-293 cell line. Cells were grown in Dulbecco's minimum essential medium–high glucose supplemented with 10 % FBS, 2 mM L-glutamine, 0.11 mg/ml Na-pyruvate, 100 U/ml penicillin-G, 0.1 mg/ml streptomycin (Invitrogen), 5 ml Non-essential Amino Acid (Sigma-Aldrich) at 37 $^{\circ}$C in a 5% CO_2 and 95% air-humidified atmosphere.

Whole-cell currents were measured in voltage-clamped cells using Axopatch 200A amplifiers connected to a personal computer using Axon Digidata 1200 data acquisition hardware (Molecular Devices Inc., Sunnyvale, CA). Series resistance compensation up to 70 % was used to minimize voltage errors and achieve good voltage-clamp conditions. Pipettes were pulled from GC 150 F-15 borosilicate glass capillaries in five stages and fire-polished, resulting in electrodes having 4–6 MΩ resistance in the bath. The bath solution contained (in mM) Choline-Cl 140, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2, $CdCl_2$ 0.1, glucose 20, and HEPES 10, at pH = 7.35. The internal solution consisted of (in mM) KCl 140, EGTA 10, $MgCl_2$ 2, and HEPES 10, at pH = 7.3. Superfusion with different test solutions was achieved using a continuous perfusion system based on gravity-flow. For data acquisition and analysis, the pClamp software package was used. Currents were low-pass filtered using the built in analog 4-pole Bessel filters of the amplifiers and sampled at 2 kHz. Before analysis, whole-cell current traces were corrected for ohmic leakage and digitally filtered (three-point boxcar smoothing).

All values presented are arithmetic means ± SEM. Statistical significance of differences was evaluated with using one-way ANOVA followed by Student's t test for paired or unpaired data, as pertinent. Differences were considered significant when the P value was

less than 0.05. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) and current version of the Hungarian Law on the Protection of Animals were strictly followed throughout the experiments.

Results

Effects of chelerythrine and bisindolylmaleimide I on I_{Kr} in canine ventricular myocytes

I_{Kr} was activated by 250 ms depolarizing pulses to +10 mV applied at a rate of 0.05 Hz. I_{Kr} was characterized as tail current amplitudes determined as the difference of the peak current and the pedestal value observed following repolarization to the holding potential of -40 mV in the presence of 5 μM nifedipine plus 1 μM HMR-1556. These I_{Kr} current tails were fully eliminated by 1 μM E-4031 indicating that the current can be considered as pure I_{Kr} mediated by hERG channels.

Exposure of myocytes to the PKC inhibitor bisindolylmaleimide I (1 μM) decreased I_{Kr} tail amplitude from 0.36±0.05 to 0.11±0.04 pA/pF ($P<0.05$, n=5, Figs. 1.A-C). Similar results were obtained with another PKC inhibitor chelerythrine (1 μM), where I_{Kr} tail current decreased from 0.39±0.04 to 0.05±0.02 pA/pF ($P<0.05$, n=4, Figs. 1.D-F). I_{Kr} was fully eliminated by 10 μM chelerythrine (n=3, not shown). In both cases the blockade of I_{Kr} developed rapidly, the maximal effect was typically achieved within a few minutes (Figs. 1.B and 1.E).

Effects of chelerythrine and bisindolylmaleimide I on hERG current in transfected HEK-293 cells

hERG current was activated by depolarization to +30 mV for 3 s, which was followed by a repolarization to -40 mV allowing for relaxation of the current resulting tail current. Amplitude of the tail current was considered as an indicator of hERG current density. In these experiments the holding potential was set to -80 mV, pulses were delivered every 30 s. Both PKC inhibitors, bisindolylmaleimide I and chelerythrine caused a concentration-dependent suppression of the hERG current (Fig. 2). This blockade was readily reversible in the case of bisindolylmaleimide I (Fig. 2.A) but only partially in the case of chelerythrine. Fitting data to the Hill equation the estimated EC_{50} values were 0.76±0.04 and 0.11±0.01 μM for bisindolylmaleimide I and chelerythrine, respectively, with the corresponding Hill coefficient of 1.21±0.07 and 1.52±0.18 (n=4 for each drug).

Both bisindolylmaleimide I and chelerythrine modified the gating kinetics of hERG current (Fig. 3). Voltage-dependence of steady-state activation was obtained by measuring tail current amplitudes at -40 mV following test pulses clamped to various voltages indicated in abscissa. Each tail current was normalized to that obtained following the most positive test potential. The voltage-dependence of activation was shifted towards more negative voltages by the two PKC inhibitors. Half-activation voltage, determined by fitting data to the two-state Boltzmann model, was shifted from 2.7±1.2 to -9.5±1.4 mV by 1 μM bisindolylmaleimide I and from 0.6±2.7 to -12.6±3.7 mV by 0.1 μM chelerythrine ($P<0.05$, n=4 for each drug). No significant changes were observed in the corresponding slope factors: 7.4±0.4 *versus* 7.2±0.3 mV^{-1} and 7.7±0.6 versus 8.1±0.5 mV^{-1}, respectively (Figs. 3.A and 3.D).

Time-dependence of activation of hERG current was determined using the tail-envelope test. Tail currents were recorded at -40 mV following test depolarizations (to

+30 mV) having increasing durations as shown in the abscissa. In the presence of bisindolylmaleimide I and chelerythrine the longest test pulse was 0.8 s, since activation was fully saturated by this time. Each tail current was normalized to that of the highest amplitude. Time constants for activation was determined by monoexponential fitting. Activation was accelerated by both compounds. Accordingly, the time constant of activation was reduced from 301±57 to 143± 22 ms and from 247±19 to 146±5 ms by 1 μM bisindolylmaleimide I and 0.1 μM chelerythrine, respectively ($P<0.05$, n=4 for each drug, Figs. 3.B and 3.E).

Time constant of deactivation was measured at -40 mV following a 3 s long depolarization to +30 mV. Deactivation was determined as a sum of two exponential components characterized by fast and slow time constants (τ_1, τ_2) and the corresponding amplitudes (A_1, A_2). 1 μM bisindolylmaleimide I significantly increased the fast and slow time constants of deactivation, while 0.1 μM chelerythrine left these time constants unaffected as indicated by Figs. 3.C and 3.F. See also the insets presented in Figs. 2.A and 2.C.

Possible role of PKC in regulation of I_{Kr} in canine ventricular cells

Although the direct blockade of hERG current by chelerythrine and bisindolylmaleimide I was demonstrated above, the effect of PKC inhibition had to be also examined. In these experiments a lower (0.1 μM) concentration of bisindolylmaleimide I, blocking PKC effectively with relatively small direct blocking action on I_{Kr}, was used. The effect of 0.1 μM bisindolylmaleimide I was tested using both low cytosolic Ca^{2+} concentration (buffered by 10 mM EGTA) and at high cytosolic Ca^{2+} level, where $[Ca^{2+}]_i$ was set to 500

nM using the Fabiato program (Fabiato and Fabiato 1979). This was designed to distinguish between possible effects on the calcium-sensitive conventional and calcium-insensitive novel PKC isoforms (Mellor and Parker 1998). As shown in Figs. 4.A and 4.B inhibition of PKC by 0.1 μM bisindolylmaleimide I failed to alter I_{Kr} tail amplitude significantly, which was independent of the actual level of Ca^{2+}_i. Similarly, I_{Kr} was not modified significantly by activation of PKC by 0.1 μM PMA in the presence of 0.5 μM cytosolic Ca^{2+} (Fig. 4.C). These results strongly suggest that PKC inhibition itself has little effect on the amplitude of I_{Kr} in canine ventricular cells.

Effects of chelerythrine and bisindolylmaleimide I on I_{Ks}

For the sake of comparison, effects of the two PKC inhibitors were studied also on I_{Ks}. I_{Ks} was activated in the presence of 5 μM nifedipine plus 1 μM E-4031 by 3 s long depolarizing pulses to +30 mV delivered at a rate of 0.1 Hz from the holding potential of -40 mV. Tail currents, obtained after repolarization, were used to characterize I_{Ks}. The current was fully eliminated by 1 μM HMR-1556, indicating that it was pure I_{Ks}.

Exposure of myocytes to bisindolylmaleimide I (1 μM) or chelererythrine (1 μM) caused small, statistically not significant, increases in I_{Ks} tail amplitudes: I_{Ks} was increased from 1.28±0.35 to 1.55±0.41 pA/pF (P=0.26, n=5) and from 1.26±0.23 to 1.41±0.32 pA/pF (P=0.58, n=6), respectively (Fig. 5).

Discussion

In the present work the effects of two frequently used PKC inhibitors, chelerythine and bisindolylmaleimide I, were studied on the rapid and slow components of the delayed

rectifier K^+ current (I_{Kr} and I_{Ks}) in canine ventricular cardiomyocytes. I_{Kr} – but not I_{Ks} – was strongly suppressed by both agents. Since the inhibitory effects of chelerythine and bisindolylmaleimide I were observed also in pure hERG channels expressed without co-expression of the members of the PKC system in HEK cells, one may conclude that these drugs block I_{Kr} directly, i.e. independently of their PKC inhibiting potencies. This is further supported by the findings that (1) the effects of chelerythine and bisindolylmaleimide I developed rapidly, and (2) manipulation of the PKC system by PMA and low concentration of bisindolylmaleimide I failed to alter I_{Kr} significantly. Although bisindolylmaleimide I has been previously reported to block hERG current directly (Thomas et al. 2004b), we are first to report a direct inhibition of hERG current and canine I_{Kr} by another PKC inhibitor, chelerythrine.

In addition to their blocking action, chelerythine and bisindolylmaleimide I caused marked changes in gating kinetics of hERG current, including a negative shift in voltage-dependence of activation, acceleration of activation and slowing of deactivation (this latter was observed only with bisindolylmaleimide I). Since these kinetic changes are incopatible with current inhibition (they are actually congruent with enhancement of the current), the blocking effect of bisindolylmaleimide I and chelerythrine is likely based on a cork-in-the-bottle mechanism of open channel block rather than being related to the observed alterations in gating kinetics.

Direct inhibition of hERG channels is not an exceptional side-effect of PKC inhibitors. The hERG-inhibitor effect of bisindolylmaleimide I has been previously described by Thomas et al. (2004b) presenting results very congruent with ours. The EC_{50} value obtained in HEK cells was 0.76 µM in our, while 1 µM in their experiments. 1 µM

bisindolylmaleimide I caused a 69.2 % inhibition in the native I_{Kr} of guinea pig (Thomas et al. 2004b) and a 69.4 % blockade of canine I_{Kr} (present study). An interesting difference between the results of the two studies can be observed in the kinetic properties of I_{Kr} blockade. We found a marked leftward shift of -12.2 mV in the voltage-dependence of I_{Kr} activation in the presence of bisindolylmaleimide I, while only a small, statistically not significant change of -2.9 mV was seen by Thomas et al. (2004b). The reason for this discrepancy is not clear at present, it might partly be caused by an improvement of voltage control due to reduction of hERG current amplitudes. However, a -20.3 mV leftward shift in steady-state inactivation was reported by Thomas et al. (2004b), which may in fact contribute to the bisindolylmaleimide I-induced I_{Kr} blockade.

In addition to I_{Kr} blockade, both chelerythrine and bisindolylmaleimide I were shown to block voltage-activated K^+ and Ca^{2+} channels in rat ventricular cells, which effect - similarly to our results – proved to be independent of PKC inhibition (Voutilainen-Myllyla et al. 2003). Acetylcholine-activated K^+ current was also blocked by chelerythrine and bisindolylmaleimide I in murine atrial myocytes (Cho et al. 2001). Furthermore, bisindolylmaleimide I inhibited voltage-dependent K^+ channels in arterial smooth muscle cells of rats and mice (Kim et al., 2004; Park et al. 2005), while another PKC inhibitor, staurosporine enhanced the activity of cardiac Na^+/Ca^{2+} exchanger in a PKC-independent manner (Kang 2008). It would be interesting to know which part(s) of these molecules are responsible for their ionotropic actions, since proper modification of these groups might result PKC inhibitors with less intensive interactions with ion channels improving thus their specificity to PKC.

Although the general conclusion of this work is that neither chelerythine nor bisindolylmaleimide I is really suitable for studying the contribution of PKC in regulation of cardiac delayed rectifier K^+ channels, some cautious remarks on this point can be made. Low concentration (0.1 μM) of bisindolylmaleimide I, which is known to block PKC effectively, while was shown in Fig. 2.B to cause approximately 10 % inhibition of hERG current, failed to decrease I_{Kr} tail amplitudes in intact canine ventricular cells (as shown in Figs. 4.A and 4.B). In contrast, there was a mild tendency of current increase during the 30 min period of superfusion. Furthermore, activation of PKC by PMA tended to slightly decrease I_{Kr} (Fig. 4.C). Since the above mentioned changes were not significant statistically, they cannot be considered conclusive. The fact, however, that the 10 % inhibition was *not* observed on canine I_{Kr} in the presence of 0.1 μM bisindolylmaleimide I suggests that PKC might moderately suppress I_{Kr} under control conditions, allowing thus the current to increase slightly upon inhibition of PKC. This is congruent with previous results on hERG channels expressed in *Xenopus* oocytes, where activation of the conventional PKC isoenzymes with thymelatoxin was shown to decrease I_{Kr} (Thomas et al. 2003).

Similarly to results obtained with I_{Kr}, a moderate, but again statistically not significant, enhancement of I_{Ks} was observed after superfusion with 1 μM chelerythrine or bisindolylmaleimide I (Fig. 5). Regarding the role of PKC in controlling I_{Ks} the published observations are quite controversial and show strong interspecies differences. For instance, the activation of PKC was shown to enhance I_{Ks} in native cardiac cells of the guinea pig (Tohse et al. 1990, Heath and Terrar 2000, Toda et al. 2007) and in oocytes expressing human I_{Ks} channel proteins (Xiao et al. 2003, Kathöfer et al. 2003,

Matavel and Lopez 2009). In contrast, I_{Ks} was suppressed by PKC activation when the oocytes were tranfected with murine or rat I_{Ks} channels (Honoré et al. 1991, Busch et al. 1992). Our results suggest that the PKC-dependent modulation of I_{Ks} in dog may be restricted to a moderate tonic inhibition, which can be suspended by blockade of the enzyme. It is interesting to note, that I_{Ks} was reduced by 20 % after superfusion with phenylephrine in canine myocytes (Robinson et al. 2000). This is in line with our findings, i.e. with the slight increase of I_{Ks} observed in the presence of PKC inhibitors (even if the difference failed to reach the level of statistical significance).

Acknowledgements

Financial support for the studies was provided by grants from the Hungarian Research Fund (OTKA-K68457, OTKA-K75904, OTKA-K73160, CNK-77855), from the Medical and Health Science Center of University of Debrecen (MEC-14/2008), and the Hungarian Government (TÁMOP-4.2.1/B-09/1/KONV-2010-007).

References

Busch AE, Kavanaugh MP, Varnum MD, Adelman JP, North RA (1992) Regulation by second messengers of the slowly activating, voltage-dependent potassium current expressed in Xenopus oocytes. J Physiol 450: 491-502

Cho H, Youm JB, Earm YE, Ho W-K (2001) Inhibition of acetylcholine-activated K^+ currentby chelerythrine and bisindolylmaleimide I in atrial myocytes from mice. Eur J Pharmacol 424:173-178

Fabiato A and Fabiato F (1979) Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (Paris) 75:463-505

Gintant G (1996) Two components of delayed rectifier current in canine atrium and ventricle. Circ Res 78:26-37

Heath BM, Terrar DA (2000) Protein kinase C enhances the rapidly activating delayed rectifier potassium current, I_{Kr}, through a reduction in C-type inactivation in guinea-pig ventricular myocytes. J Physiol 522:391-402

Honoré E, Attali B, Romey G, Heurteaux C, Ricard P, Lesage F, Lazdunski M, Barhanin J (1991) Cloning, expression, pharmacology and regulation of a delayed rectifier K^+ channel in mouse heart. EMBO J 10: 2805-2811

Kang TM (2008) PKC-independent stimulation of cardiac Na^+/Ca^{2+} exchanger by staurosporine. Korean J Physiol Pharmacol 12:259-265

Kathöfer S, Röckl K, Zhang W, Thomas D, Katus H, Kiehn J, Kreye V, Schoels W, Karle C (2003) Human $beta_3$-adrenoreceptors couple to KvLQT1/MinK potassium

channels in Xenopus oocytes via protein kinase C phosphorylation of the KvLQT1 protein. Naunyn Schmiedebergs Arch Pharmacol 368:119-126

Kim AK, Bae YM, Kim J, Kim B, Ho W-K, Earm YE, Cho SI (2004) Direct block by bisindolylmaleimide of the voltage-dependent K^+ currents of rat mesenteric arterial smooth muscle. Eur J Pharmacol 483:117-126

Li G-R, Feng J, Yue L, Carrier M, Nattel S (1996) Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. Circ Res 78:689-696

Magyar J, Bányász T, Szigligeti P, Körtvély Á, Jednákovits A, Nánási PP (2000) Electrophysiological effects of bimoclomol in canine ventricular myocytes. Naunyn Schmiedeberg's Arch Pharmacol 361:303-310

Matavel A, Lopes CM (2009) PKC activation and PIP_2 depletion underlie biphasic regulation of I_{Ks} by Gq-coupled receptors. J Mol Cell Cardiol 46:704-712

Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332:281-292

Park WS, Son YK, Ko EA, Ko JH, Lee HA, Park KS, Earm YE (2005) The protein kinase C inhibitor, bisindolylmaleimide (I), inhibits voltage-dependent K^+ channels in coronary arterial smooth muscle cells. Life Sci 77:512-527

Robinson RB, Liu QY, Rosen MR (2000) Ionic basis for action potential prolongation by phenylephrine in canine epicardial myocytes. J Cardiovasc Electrophysiol 11:70-76

Szabó G, Szentandrássy N, Bíró T, Tóth IB, Czifra G, Magyar J, Bányász T, Varró A, Kovács L, Nánási PP (2005) Asymmetrical distribution of ion channels in canine and human left ventricular wall: epicardium versus midmyocardium. Pflügers

Arch 450:307-316

Szentadrássy N, Bányász T, Bíró T, Szabó G, Tóth B, Magyar J, Lázár J, Varró A, Kovács L, Nánási PP (2005) Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. Cardiovasc Res 65:851-860

Thomas D, Zhang W, Wu K, Wimmer AB, Gut B, Wendt-Nordal G, Kathöfer S, Kreye VA, Katus HA, Schoels W, Kiehn J, Karle CA (2003) Regulation of HERG potassium channel activation by protein kinase C independent of direct phosphorylation of the channel protein. Cardiovasc Res 59:14-26

Thomas D, Wu K, Wimmer AB, Zitron E, Hammerling BC, Kathöfer S, Lueck S, Bloehs R, Kreye VA, Kiehn J, Katus HA, Schoels W, Kübler W, Karle CA (2004**a**) Activation of human ether-a-go-go related gene potassium currents is regulated by alpha(1A)-adrenoceptor. J Mol Med 82:826-837

Thomas D, Hammerling BC, Wimmer AB, Wu K, Ficker E, Kuryshev YA, Scherer D, Kiehn J, Katus HA, Schoels W, Karle CA (2004**b**) Direct block of hERG potassium channels by the protein kinase C inhibitor bisindolylmaleimide I (GF109203X). Cardiovasc Res 64:467-476

Toda H, Ding WG, Yasuda Y, Toyoda F, Ito M, Matsuura H, Horie M (2007) Stimulatory action of protein kinase C epsilon isoform on the slow component of delayed rectifier K^+ current in guinea-pig atrial myocytes. Br J Pharmacol 150:1011-1021

Tohse N, Kameyama M, Sekiguchi K, Shearman MS, Kanno M (1990) Protein kinase C activation enhances the delayed rectifier potassium current in guinea-pig heart

cells. J Mol Cell Cardiol 22:725-734

Voutilainen-Myllyla S, Tavi P, Weckström M (2003) Chelerythrine and bisindolylmaleimide I prolong cardiac action potentials by protein kinase C-independent mechanism. Eur J Pharmacol 466:41-51

Xiao GQ, Mochly-Rosen D, Boutjdir M (2003) PKC isozyme selective regulation of cloned human cardiac delayed slow rectifier K current. Biochem Biophys Res Commun 306:1019-1025

Figure legends

Fig. 1. Effects of the PKC inhibitor bisindolylmaleimide I (Bim I, **A-C**) and chelerythrine (**D-F**) on I_{Kr} in canine ventricular myocytes. **A, D**: Representative superimposed I_{Kr} tails obtained before and after drug exposure. **B, E**: Time course of development of drug effects. **C, F**: Average I_{Kr} tail amplitudes in control and in the presence of 1 μM bisindolylmaleimide I (n=5) and 1 μM chelerythrine (n=4). Columns and bars indicate means ± SEM values, asterisks denote significant ($P<0.05$) differences from control values.

Fig. 2. Concentration-dependent effects of bisindolylmaleimide I (Bim I, **A, B**) and chelerythrine (**C, D**) on hERG current expressed in HEK-293 cells. **A, C**: Representative traces showing superimposed hERG current records obtained in control, in the presence of cumulatively increasing concentrations of bisindolylmaleimide I or chelerythrine, as indicated, and following washout. Insets: I_{Kr} tail current tails obtained in the presence 1 μM bisindolylmaleimide I and 0.1 μM chelerythrine were magnified so as their amplitudes should be identical to that of the corresponding control traces. **B, D**: Cumulative dose-response curves. Solid lines were obtained by fitting data to the Hill equation. Symbols and bars indicate means ± SEM values, collected from 4 cells with each drug.

Fig. 3. Effects of 1 μM bisindolylmaleimide I (Bim I) and 0.1 μM chelerythrine on the kinetic properties of hERG current. **A, D**: Voltage-dependence of steady-state activation.

Results were fitted to the Boltzmann function to estimate the half-activation voltage ($V_{0.5}$) and slope factor. **B, E**: The time-dependence of activation of hERG current determined using the tail-envelope test. Solid lines were obtained by monoexponential fitting. **C, F**: Deactivation of hERG current at -40 mV was determined as a sum of two exponential components characterized by a fast and a slow time constant (τ_1 and τ_2) and the corresponding amplitudes (A_1 and A_2). Symbols, columns and bars indicate means ± SEM values obtained in 4 cells with each drug, asterisks denote significant ($P<0.05$) differences from control.

Fig. 4. Time-dependent effects of low concentrations of bisindolylmaleimide I (Bim I) and PMA on I_{Kr} tail amplitude in canine ventricular cells. **A**: Effect of 0.1 μM bisindolylmaleimide I in the presence of low cytosolic calcium buffered by 10 mM EGTA in the pipette solution (n=6). **B**: Effect of 0.1 μM bisindolylmaleimide I in the presence of high (0.5 μM) cytosolic Ca^{2+} concentration set using the Fabiato program (n=5). **C**: Effect of 0.1 μM PMA in the presence of high cytosolic Ca^{2+} (n=5). I_{Kr} tails were normalized to their respective initial values. Symbols and bars are means ± SEM, dotted lines indicate the baseline level.

Fig. 5. Effects of bisindolylmaleimide I (**A-C**) and chelerythrine (**D-F**) on I_{Ks} in canine myocytes. **A, D**: Representative superimposed I_{Ks} tails obtained before and after drug exposure. **B**, **E**: Time course of development of drug effects. **C, F**: Average I_{Ks} tail amplitudes in control and in the presence of 1 μM bisindolylmaleimide I (n=5) or 1 μM chelerythrine (n=6). Columns and bars indicate means ± SEM values.